

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34302 A2

- (51) International Patent Classification⁷: **B01L 3/00** Choong, Vi-En [MY/US]; 3380 West Genoa Way, Chandler, AZ 85226 (US).
- (21) International Application Number: PCT/US00/42047
- (22) International Filing Date: 9 November 2000 (09.11.2000) (74) Agent: SILVA, Robin, M.; Flehr, Hohbach Test Albritton & Herbert LLP, Four Embarcadero Center, Suite 3400, San Francisco, CA 94111 (US).
- (25) Filing Language: English (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (26) Publication Language: English
- (30) Priority Data: 09/438,600 12 November 1999 (12.11.1999) US (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application: US 09/438,600 (CON) Filed on 12 November 1999 (12.11.1999)
- (71) Applicant (*for all designated States except US*): MOTOROLA, INC. [US/US]; 1303 East Algonquin Road, Schaumburg, IL 60096 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): SHIEH, Chan-Long [US/US]; 6739 East Bar Z Lane, Paradise Valley, AZ 85253 (US). FOLEY, Barbara [US/US]; 14842 South Foxtail Lane, Phoenix, AZ 85048 (US). YU, Huinan [US/US]; 5760 West Park Avenue, Chandler, AZ 85226 (US).
- Published:
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 01/34302 A2

(54) Title: BIOCHANNEL ASSAY FOR HYBRIDIZATION WITH BIOMATERIAL

(57) Abstract: The invention relates to a microfluidic device with microchannels that have separated regions which have a member of a specific binding pair member such as DNA or RNA bound to porous polymer, beads or structures fabricated into the microchannel. The microchannels of the invention are fabricated from plastic and are operatively associated with a fluid propelling component and detector.

Biochannel Assay for Hybridization with Biomaterial

5

BACKGROUND OF THE INVENTIONFIELD OF THE INVENTION

10 The invention pertains to the structure, fabrication of a microfluidic device and methods for conducting analysis in microfluidic devices.

BACKGROUND OF THE ART RELATED TO THE INVENTION

 Analysis utilizing specific binding pairs such as antigen/antibody; complementary
15 DNA/DNA; DNA/RNA; RNA/RNA; biotin/avidin containing pairs are widely known in the art. Techniques for manufacturing and utilizing microfluidic devices are also well known. The art also discloses various techniques for DNA sequencing based on complementary binding of DNA.

20 DNA probe array technology, which utilizes binding of target single stranded DNA onto immobilized DNA probes has wide applications. A large amount of research and development activities have been carried out with different technology emphasis. For example, same technologies are focused on probe placement by mechanical means. Other technologies are focused on in-situ probe synthesis that is advantageous in producing large arrays.
25 Additionally, other technologies are focused on gel pad arrays using photopolymerization and piezoelectric liquid dispensing technologies.

 A common challenge to all DNA hybridization technologies is the lack of control of stringency for each individual probe site. The DNA hybridization process occurs at specific
30 temperature and salinity conditions and varies with DNA sequences. For DNA probe arrays,

since the DNA probe sequences are different, hybridization recognition is never perfect under a uniform stringency condition for the entire probe array. The problem is most obvious for short duplexes which often results in single base mismatches. One can minimize the effect of mismatched hybridization by using large probe site redundancy. Stringency control has been
5 provided for each probe site by controlling the electrophoretic movement of oligonucleotides. To successfully implement this later scheme, a meticulously engineered permeation layer is required to prevent DNA molecules or labeling agents being damaged by direct electrolysis or by the product of the electrolysis.

10 In addition, the current DNA array technologies have failed to provide an effective solution to maximize hybridization efficiency. For diagnostic assays, the target DNA molecules are often of minute quantities. The detection limit of the assay is determined by the sensitivity of the detection device, and also by the amount of target oligos bound to the probes during the course of hybridization. In a stationary hybridization chamber where active mixing
15 is absent, the probability of a given target molecule hybridizes to its complementary strand on the surface is determined by diffusion rate and statistics. It takes up to tens of hours for hybridization to complete at low target concentration levels. To better utilize the target molecules and enhance the hybridization, flow through technology has been proposed where the probe arrays are placed perpendicular to the fluidic flow direction. Even with flow through
20 technology, only a portion of the target molecules can come in contact with any specific DNA probe site.

The present invention overcomes the above technical issues by sequentially placing the DNA probe sites in microfluidic channels such that the DNA probe can efficiently contact its
25 binding partner.

U.S. Patent 5,147, 607 describes a variety of microassay devices which have microchannels in plastic materials with a reagent such as an antibody or DNA immobilized on the channel at different locations. Techniques for binding antibodies to the microchannel wall are described but techniques for binding DNA are not described. The binding of probes to the microchannel wall does not provide for optimum contact of probe and test sample. U.S. Patent 5,843,767 describes microfabricated flowthrough porous apparatus for discrete detection of binding reactions such as DNA/DNA. WO/98/43739 describes porous flow channels having reagents immobilized in the chamber.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic top view of a fluid channel filled with porous gel and spotted DNA probes.

Figure 2 shows lithographically patterned gel pads inside a microfluidic channel.

Figure 3 shows microfluidic channels with molded plastic microstructures for DNA attachment.

Figure 4 shows a microfluidic channel packed with beads where distinct sections of beads have a specific binding agent such as DNA.

Figure 5 illustrates a simple initial flow being directed into numerous channels.

Figure 6 illustrates a circulating microfluidic channel device.

SUMMARY OF THE INVENTION

The invention comprises microfluidic devices comprising a section of solid material such as a chip with a microchannel with an inlet and exit port for flowing fluids through the channels. The microchannel has separated defined regions of specific binding pair members immobilized on porous polymer, microstructures molded in the microchannels or packed beads. These structures provide for optimum contact of the immobilized binding pair member and a binding pair member in fluid flowing through the microchannel. The porous polymer beads or microstructure must provide for flow and not obstruct the channel. The microchannel is operatively associated with a detector and a fluid propelling component to flow liquids in the channel and may also have electrodes at the exit and entrance ports.

DNA/DNA; DNA/RNA, and RNA/RNA complementary binding pairs are preferred. The microchannel is operatively associated with target DNA labeled with a fluorophore, an excitation source and a detector to detect emitted fluorescence from the binding pairs. It is an object of the invention to provide a method for DNA or RNA sequencing by providing the above identified chip with DNA or RNA probes immobilized in the separated defined region to bind fluorescently labeled target DNA.

It is also an object of the present invention to provide a means for determining genetic defects. The invention also provides a means for identifying pathogens through DNA analysis.

The microchannels may have a variety of configurations, feedback arms, valves, and vents to control fluid flow. There may be single or multiple channels. The invention provides for efficient contact between immobilized binding substances and binding partners in the fluid flowing through the channel. The invention provides for improved hybridization stringency

control by flow modulation; shortened assay time by increasing the rate of hybridization with flow induced agitation and by bringing the target and probe into proximity within the microfluidic channel; and increased hybridization efficiency which improves sensitivity. In addition there is no interference through hydrolysis.

DETAILED DESCRIPTION OF THE INVENTION

The microfluidic channels of the present invention are channels generally less than 200 microns in plastic with molding or embossing technology. The channels need to be of the dimension to support pumping of the microfluidic system. The microfluidic channel may have any shape, for example, it may be linear, serpentine, arc shaped and the like. The cross-sectional dimension of the channel may be square, rectangular, semicircular, etc. There may be multiple and interconnected microchannels with valves to provide for recirculation.

The section of solid material may be chips made of glass, ceramic, metal, silicon or plastic. Chips are preferably fabricated from plastics such as epoxy resin, polyacrylic resins, polyester resins, polystyrene, polycarbonate, polyvinyl chloride and the like. Specific binding pairs are DNA/DNA or DNA/RNA complementary binding pairs.

Fluid propelling components such as pressurized gas, vacuum, electric field, magnetic field and centrifugal force devices are operatively associated with the microchannel to move fluid through the microchannel. In addition, charged test samples may be altered by modulating the electric field against or in the direction of the flow or perpendicular to the flow. Thus, the rate of fluid flow in the microchannel can be altered to promote binding of binding pairs, for example, hybridization of DNA/DNA or DNA/RNA pairs. Also, operatively associated with the microchannel is a detector such as an optical, electrical or electrochemical detector.

Figure 1 illustrates a serpentine shaped microfluidic channel 1 filled with porous gel 2 with discrete separate regions 3 which have attached a member of a specific binding pair, such as DNA. Sample flows into the microfluidic channel at 4 and exits the channel at 5. In this approach, the channel is filled with porous gel material such as agarose or polyacrylamide. The pores of the gel are made large enough by using dilute gelling solutions to permit significant

fluid flow through the gel. Members of specific binding pairs are spotted onto the gels so that the probes are chemically attached.

Figure 2 illustrates a microfluidic channel 10 which has patterned gel pads 11 within
5 the channel. The gel pads are formed by photopolymerization of acrylamide using lithographic techniques.

Figure 3 illustrates a microfluidic channel 15 where high surface area microstructures are molded into the channel. Figure 3a shows a series of columns 16 in a distinct region and
10 Figure 3b shows a distinct region of domes 17 molded into channel 15. These microstructures are chemically modified and specific binding substances are attached.

Figure 4 illustrates a microfluidic channel 20 packed alternately with regions of plain beads 21 and beads 22 having a specific binding substance, such as DNA.

15 Figure 5 illustrates a microfluidic channel 25 which branches in multiple microfluidic channels 26 a, b, c etc each of which have a distinct region of a binding substance 27 as described above. Through this embodiment, a sample can be studied in parallel to test its reactivity to the same or different specific binding substance.

20 Figure 6 illustrates a chip 30 with a recirculating microfluidic channel 34. The microfluidic channel has discrete areas with specific binding substances 32 as described above and a recirculating arm 33 and a valve 34 for output after recirculation.

In this embodiment the test sample is recirculated past the location of the binding partner.

25 Thus, dilute samples or slow reacting samples can be respectively passed by the specific binding substance.

Microfabricated plastic capillary electrophoresis (CE) devices have been demonstrated in the art. Thermoplastic molded polymethylmethacrylate CE devices are described by R.M. McCormick, et al, "Microchannel electrophoretic separations of DNA in injection-molded plastic substrates," *Anal. Chem.*, vol. 69, pp. 2626, 1997. Eckstrom *et al* investigated
5 elastomeric polymers such as PDMS, "PCT Appl. WO91/16966," 1991. More recently, others have published electrophoretic separation of DNA ladders in PDMS devices, for example, C.S. Effenhauser, et al, "Integrated Capillary Electrophoresis on Flexible Silicone Microdevices," *Anal. Chem.*, vol. 69, pp.3451, 1997. Mastrangelo, et al describes building micro CE devices based on parylene-polycarbonate substrates using a surface micromachining approach, "An
10 Inexpensive Plastic Technology for Microfabricated Capillary Electrophoresis Chip" presented at Micro-TAS'98, Banff, 1998. Thus, techniques are available for fabricating microchannels. The invention involves fixing specific binding substances by way of porous polymer, beads or structure in the microchannel to more efficiently promote binding.

These examples are intended to illustrate the present invention and not to limit it in spirit
15 or scope.

What is claimed:

1. A microfluidic device comprising a section of solid material with a microchannel having an entrance and exit port for the transportation of fluids in and out of the microchannel wherein the microchannel contains spacially separated defined regions of specific
5 binding pair member immobilized on a porous polymer, beads or on microstructures fabricated in the microchannel.

2. The microfluidic device of claim 1 wherein the device is fabricated from silicon, glass, silicon dioxide, plastic or ceramics.

10

3. The microfluidic device of claim 1 wherein the separated defined regions are porous polymer with specific binding pair member bound to the porous polymer.

4. The microfluidic device of claim 1 wherein the separated defined regions have
15 beads with specific binding pair member bound to the bead.

5. The microfluidic device of claim 1 wherein the defined regions are with immobilized binding pair members are formed by introducing hydrogels in the microchannels.

20 6. The defined region of claim 5 wherein the binding pair members are selectively dispensed on spatially separated portions of hydrogel.

7. The defined region of claim 5 wherein the hydrogels in the microchannels are patterned by means including photolithography.

25

8. The microfluidic device of claim 1 wherein the separated defined regions have microstructures fabricated into the microchannel and the microstructures have specific binding pair member bound thereto.

5 9. The microfluidic device of claim 1 wherein the binding pair members from a group consisting of DNA, RNA, polypeptides, nucleic acids, and antibody/antigens.

10 10. The microfluidic device of claim 1 wherein the specific binding member is a DNA or RNA probe.

11. The microfluidic device of claim 1 wherein the specific binding member is DNA.

12. The microfluidic device of claim 1, further comprising a fluid propelling
15 component that is operatively associated with the microchannels.

13. The fluid propelling component in claim 12 is a pressurized gas, vacuum, electrical field, magnetic field or centrifugal force.

20 14. The microfluidic device of claim 1, comprising a detector component that is operatively associated with the microchannels.

15 15. The microfluidic device of claim 14 wherein the detector is an optical, electrical or electrochemical detector.

16. A method of detecting a specific binding member in a test sample comprising:
- a. providing the microfluidic device of claim 1;
 - b. flowing the test sample through the microchannel to form a binding pair;
 - c. detecting the binding pair.

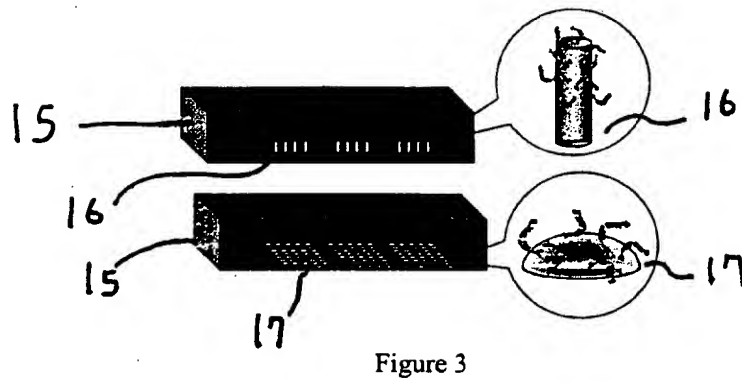
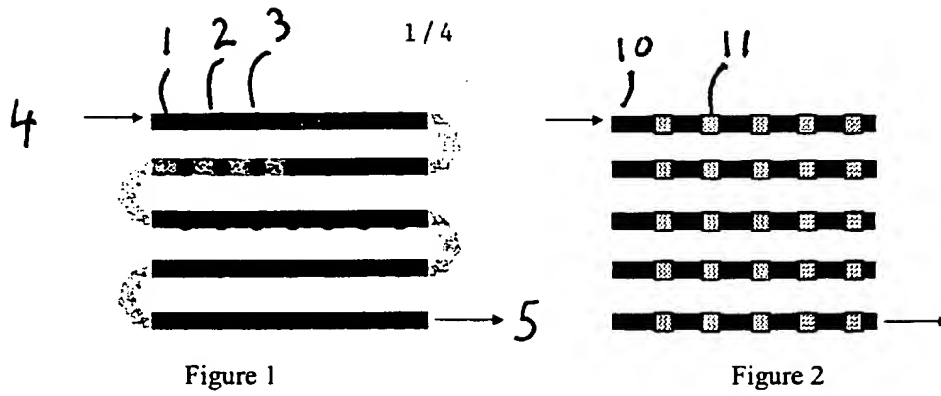
5

17. The method of claim 16 wherein the flow of the test sample is recirculated in the microchannel.

18. The method of claim 16 wherein the flow rate of the test sample is adjusted by a
10 fluid propelling component operatively associated with the microchannel.

19. The method of claim 16 wherein the migration speed of a charged sample is further modified through applying a modular electrical field in or against the direction of flow.

15 20. The method of claim 16 wherein the charged test sample is attracted or repelled at the spatially defined region by the application of electrical field in the direction perpendicular to the flow direction.



BEST AVAILABLE COPY

BEST AVAILABLE COPY

2/4

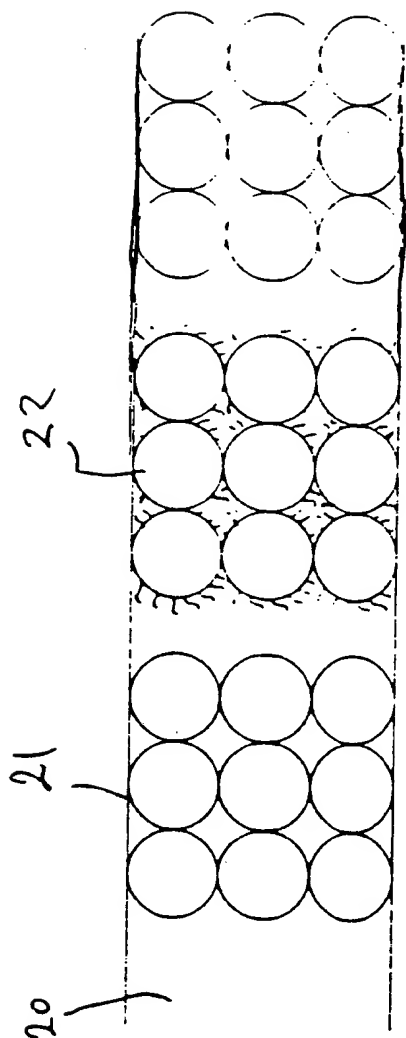


FIGURE 4

3/4

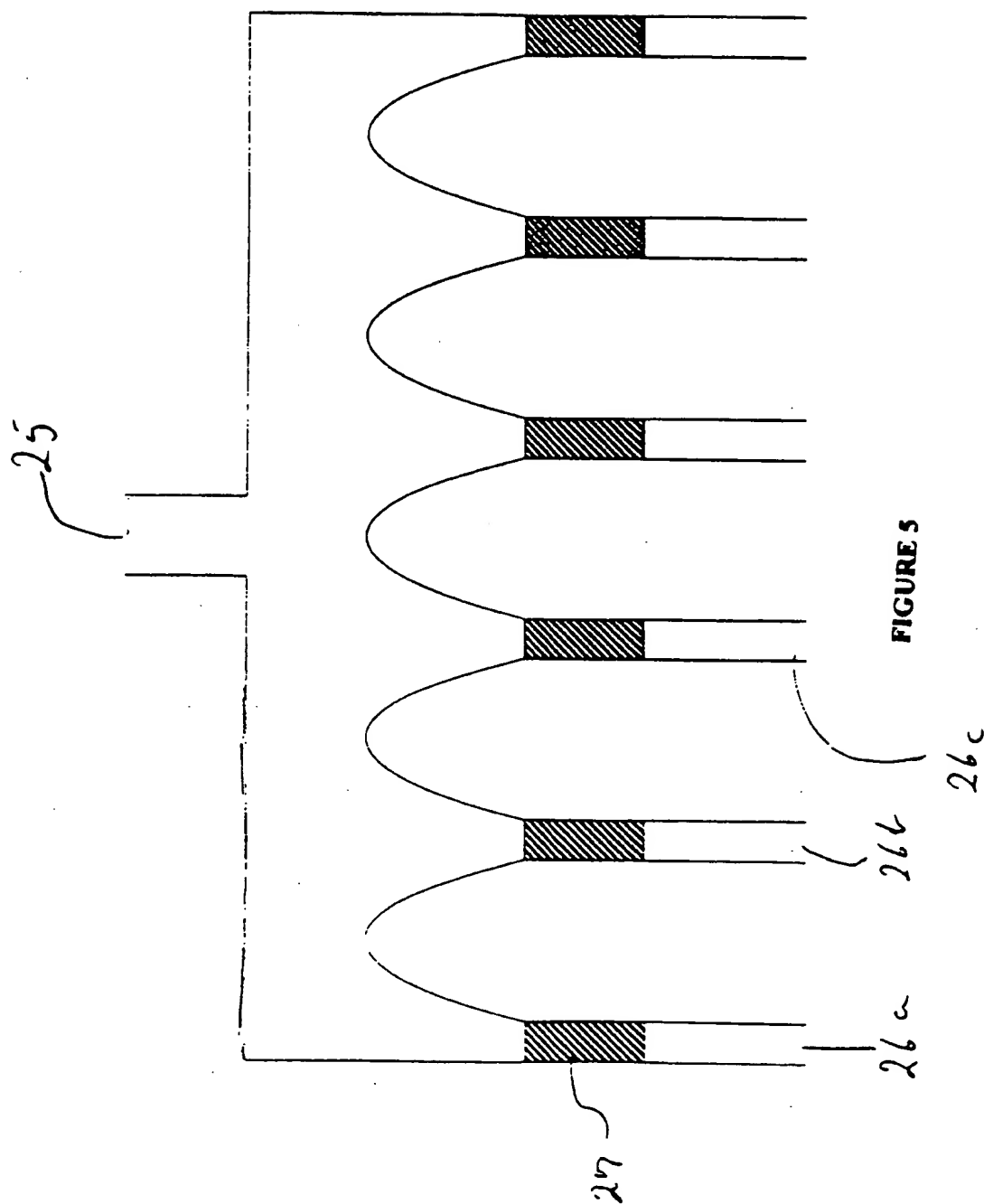
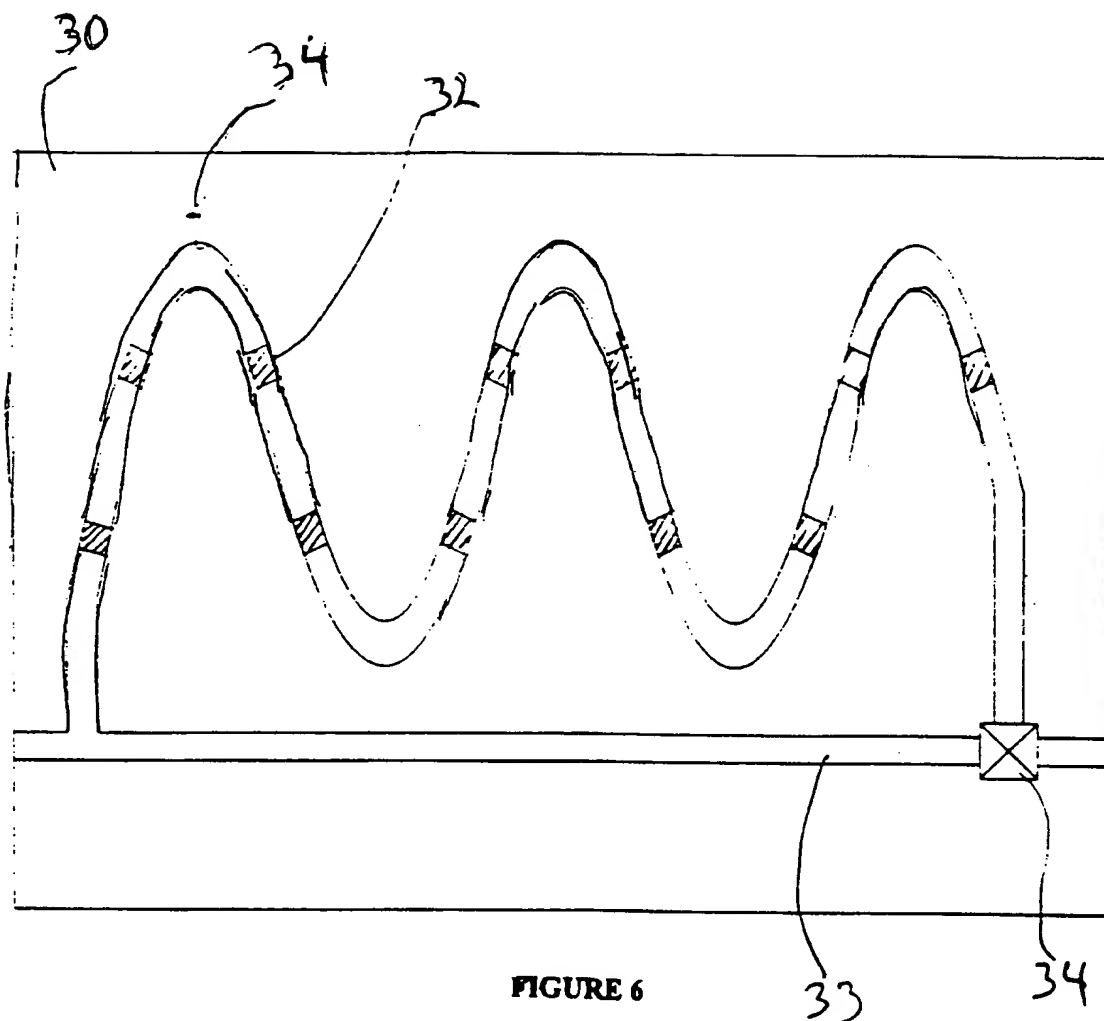


FIGURE 3

4/4



BEST AVAILABLE COPY